THROMBOPLASTIN GENERATION TEST IN NORMAL HORSES AND HORSES INJECTED WITH TETANIC TOXIN *

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It seems that Zimmermann (15) was the first author to call the attention to a possible slowness of the process of blood coagulation in the horse. From then onward, several researchers demonstrated differences in the phenomenon in the equine species when compared to the human species, although Soulier and Larrieu (14) declared that the concentration of coagulation factors are similar in the two species.

The greatest difficulties are found in the factors that enter in the formation of thromboplastin. Bell et col. (2, 3) believe that there is deficiency of anti-hemophilic factor in the horse plasma, which was confirmed by Barkhan et col. (1). On the other hand Sjolin (12) first admitted deficiency of the Christmas factor but later on (13) he thought of a probable insuficiency of a factor similar or identical to the Hageman factor.

Fantl e Marr (7) found a significant quantitative difference between the thromboplastinic factors of horse and human blood, although their activities were similar. Fantl and Ward (8) also observed a low activity in the thromboplastic component of the horse platelets.

Therefore, we thought it would be useful to make a comparative analysis of the activity of the thromboplastinic factors, expressed by the thromboplastin generation test in equine and human plasma. Another investigation was about an eventual difference in the generating activity of thromboplastin among normal animals and injected with tetanic toxin, and also whether there is an optimum time in the thromboplastin generation test.

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Material and methods

20 normal equines were used, 14 male castrated horses and 6 female, and 10 male horses, also castrated, which had been used for the production of anti-tetanic serum. The age of the animals was between 10 to 20 years, all belonging to Instituto Butantan, and they were submitted to one same diet. The blood was collected with 10% of 0.1 M sodium oxalate from the jugular vein, while the animals were resting and a siliconized syringe was used. The plasma was obtained by centrifugation of blood for 10 minutes at 2.500 r.p.m.

The thromboplastin generation test was performed according to the method of Biggs and Douglas (1) using a platelet suspension of the own animal as a source of platelet-thromboplastinic factors. The citrated plasma of the original technique was substituted by the oxalated plasma absorbed by barium sulphate, washed according to the technique described by Biggs and Macfarlane (5) used in a proportion of 0.1 g of the salt for 1 ml of plasma. The absorption was made at 37°C for 30 minutes, shaking the tubes once in a while. Afterwards, they were centrifuged at 2.500 r.p.m. for 10 minutes; the supernatant was removed and diluted 1:5 to be used.

The platelets for the suspension were obtained from blood collected in 10% of a 3.8% saline diluted sodium citrate. They were washed three times in saline, finally suspended in the same solution according to a concentration about 3 times the one of the plasma.

The test lasted 8 minutes for the normal animals and 5 for the animals injected with tetanic toxin. In the first case the readings were performed every minute, during the 8 minutes, and in the second case, from the second to the fifth minute. Two generation tests were done for each animal.

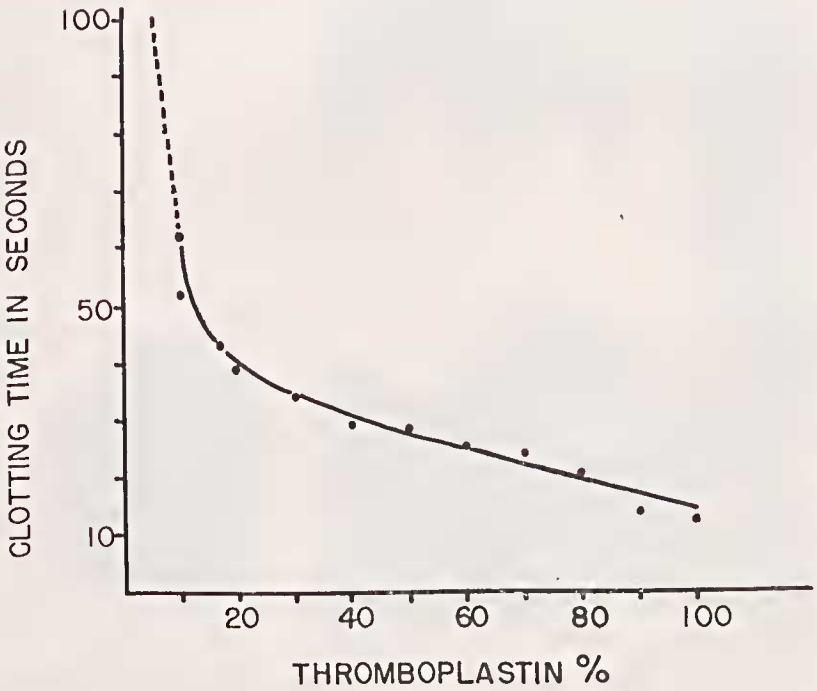
For transformation of data in concentration a thromboplastin dilution curve obtained with human plasma was used (figure 1). The values obtained in the two experiments, were transformed in concentration and the average, which represented the results for each animal, was calculated.

The human values, used as comparison, consisted of the results obtained in the Department of Physiopathology of the Instituto Butantan, with 10 individuals, both male and female and of different ages, using their own platelets with a similar technique and analysed according to the same dilution curve.

The level of rejection adopted for a statistical comparative analysis of the groups was 5%.

FIGURE I

REFERENCE FIGURE OF HUMAN THROMBOPLASTINIC ACTIVITY WITH PLATELETS SYSTEM IN RELATION TO TIME IN SECONDS AND PERCENTAGE OF CONCENTRATION.



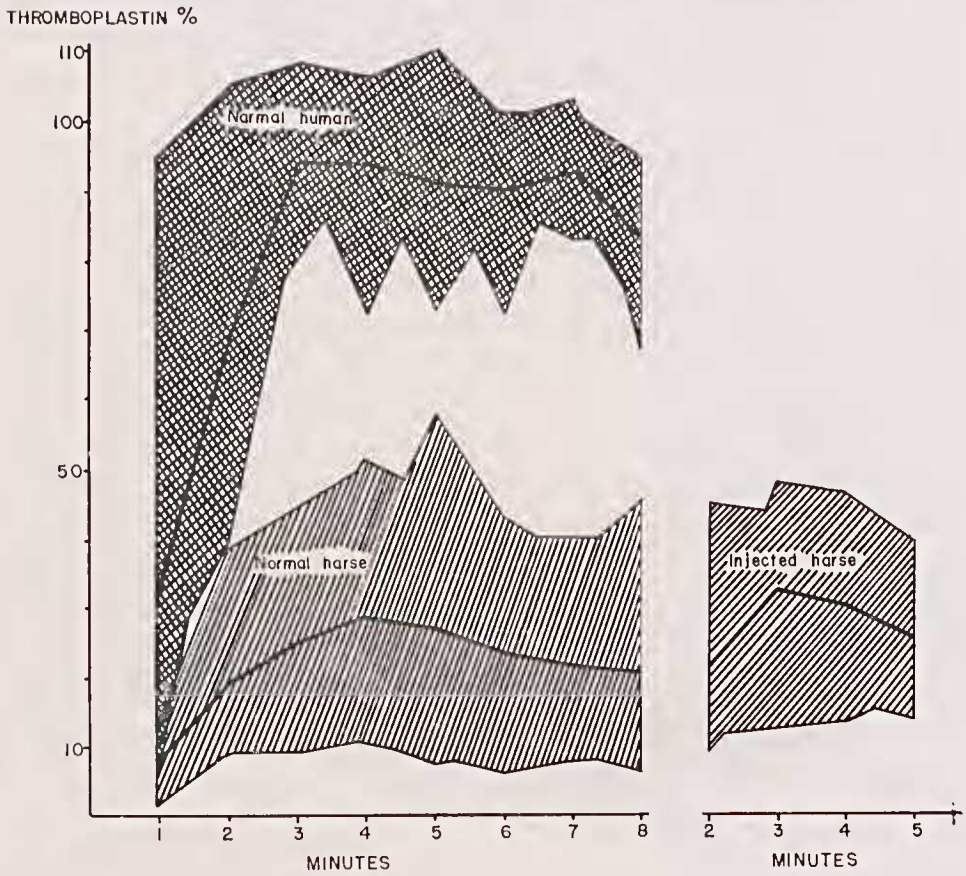
Results

The results obtained, are summarized in table I. This table shows the mean values, standard deviation, median, Pearson's coefficient of variability and the estimations of 95% confidence intervals for the average, of the minute in which the maximum thromboplastin generation and also the maximum activity of the generated thromboplastin occurred. It was revealed by the clotting time of the substrate plasma by the action of calcium chloride and of the system in that same minute.

Figure 2 illustrates the distribution of frequencies for each tested group, showing also the mean curves of thromboplastin generation.

FIGURE II

FREQUENCY DISTRIBUTION OF T.G.T. CURVES OF NORMAL HUMAN PLASMA, NORMAL HORSE PLASMA AND PLASMA OF HORSES INJECTED WITH TETANIC TOXIN.



Discussion

It was already demonstrated that the coagulation system of the equines shows differences from that of the man, in what regards several different factors (6, 9, 11). However, more attention was given to the thromboplastinic factors, because it is in relation to them that most opinions differ. While some (1, 2, 3) admit that there is deficiency of anti-hemophilic globulin in horse plasma, when it is compared to that of man, others admit deficiency of Christmas (12) or even Hageman (13) factor. What really seems to exist is a quantitative difference of factors in the two species (7) and not a qualitative one. Anyway, when both species are compared, a smaller activity of the thromboplastin generation system of the blood of equines seems to be evident, when measured altogether.

TABLE I — SUMMARY OF THE RESULTS OBTAINED IN THROMBOPLASTIN GENERATION TEST FOR MAN, NORMAL HORSES, AND INJECTED WITH TETANIC TOXIN, CLASSIFIED ACCORDING TO THE TESTS PERFORMED AND OF THE MEASURES OF POSITION AND CALCULATED VARIABILITIES

	Minute of maximum generation			Maximum activity in % of clotting time		
	Human	Normal horse	Injected horse	Human	Normal horse	Injected horse
Average	3,6	3,4	3,2	99,2	31,7	37,2
Standard deviation	0,69	1,01	0,92	6,3	14,92	10,5
Median	3,5	3,5	3,0	99,0	33,5	42,7
Pearson's coef. of variab. % .	19,16	29,24	28,75	6,44	47,06	28,44
95% confidenee interval for average	3,07 to 4,12	2,96 to 3,93	2,51 to 3,89	94,38 to 104,01	24,54 to 38,86	29,23 to 45,17

The analysis of our results shows a wide line of transvariation with the intervals of confidence calculated for the minute in which the greatest thromboplastin activity is developed in relation to the human plasma, normal equine plasma and plasma of horses injected with the tetanic toxine, thus demonstrating that there is no significant difference for the level of rejection adopted. Therefore, we can conclude that the time which is necessary for this activity to develop is the same for the man and horse, between the third and fourth minute, as was observed by Martins (10).

In relation to the maximum activity of the generated thromboplastin, the same transvariation is verified between the normal horses, and the ones injected with tetanic toxin, however the confidence interval for these two groups of animals is quite different from the one found for man, permitting us to assume that the activity of the equine thromboplastin is much smaller than that of the man (figure 2).

Therefore it is not necessary to make determinations before the second and after the fifth minute, in the thromboplastin generation test of Biggs and Douglas.

Summary

Normal horses have a smaller amount of thromboplastin than normal humans when measured by the thromboplastin generation test with the method of Biggs and Douglas.

Horses injected with tetanic toxin for the preparation of hyperimmune serum have the same thromboplastin activity as the normal animals.

The maximum thromboplastin generation is observed at the third or fourth minute with the method of Biggs and Douglas, in normal horses and in horses injected with tetanic toxin, and are the same times observed in normal humans.

Resumo

Cavalos normais têm menor quantidade de tromboplastina do que o homem normal quando medida pelo teste de geração de tromboplastina, método de Biggs e Douglas.

Cavalos injetados com toxina tetânica para preparação de sôro hiperimune, têm a mesma atividade tromboplastínica que os animais normais.

O máximo de geração de tromboplastina é observado no terceiro ou quarto minuto com o método de Biggs e Douglas, tanto nos cavalos normais como nos injetados com toxina tetânica e êste tempo é o mesmo que o observado no homem normal.

References

1. *Barkhan, P., Tomlin, S. C.* and *Archer, R. K.* — Comparative coagulation studies on horse and human blood. *J. Comp. Path.*, **67**:358, 1957.

2. *Bell, W. N., Archer, R. K.* and *Tomlin, S. C.* — Coagulation mechanism of the horse. *Nature* **175**(4457):596, 1955.

3. *Bell, W. N., Tomlin, S. C.* and *Archer, R. K.* — The coagulation mechanism of the blood of horse with particular reference its "haemophilioid" status. *J. Comp. Path.*, **65**:255, 1955.

4. *Biggs, R.* and *Douglas, A. S.* — The thromboplastin generation test. *J. Clin. Path.*, **6**:23, 1953.

5. *Biggs, R.* and *MacFarlane, R. G.* — *Human blood coagulation and its disorders.* 2nd ed., Oxford, Blackwell Scientific Publications, 1957, 388.

6. *Burstein, M.* and *Guinand, A.* — Sur la specificité du fibrinogène des mammifères. I — Temps de thrombine du plasma de quelques mammifères. Rôle du pH et du calcium. *Rev. Hemat.*, **9**:231, 1954.

7. *Fantl, P.* and *Marr, A. G.* — The coagulation of the horse blood. *J. Physiol.*, **142**:197, 1958.

8. *Fantl, P.* and *Ward, H. A.* — The thromboplastic component of intact blood platelets is present in masked form. *Australian J. Exper. Biol. Med. Sci.*, **36**:449, 1958.

9. *Kment, A.* — Beitrag zur "Plasmagerinnungszeit" der Haustiere. *Wien. Tierärztl. Wchnschr.,* **37**:461, 1950.

10. *Martins, L. F.* — *Contribuição ao estudo do coagulograma do cavalo puro sangue inglês.* Tese de doutoramento, Fac. Med. Veterinária, São Paulo, 1962.

11. *Quick, A. J.* — The prothrombin concentration in the blood of various species. *Amer. J. Physiol.,* **132**:239, 1941.

12. *Sjolin, K. E.* — Laek of Christmas factor in horse plasma. *Nature,* **178**(4525): 153, 1956.

13. *Sjolin, K. E.* — Coagulation defect in horse plasma. *Proc. Soc. exp. Biol.,* **94**: 818, 1957.

14. *Soulier, J. P.* and *Larrieu, M. J.* — Measurements of thromboplastie factors and profactors in plasma. I. Deficits in thromboplastin. Study of reagents, measurements of anti-hemophilie and of platelet activities. *J. Lab. Clin. Med.,* **41**:849, 1953.

15. *Zimmermann* — 1847 — Cit. *Sopeña, I.* — Determinación del número normal de plaquetas sanguineas en algunas espécies domésticas. *Rev. Fac. Agron. B. Aires,* **9**:1947.